Remarks:

Please find remarks directed towards the individual points of the examiner's detailed action of the 08/14/2004 office action itemized below:

Non-Claim Objections:

2.) According to the action, the priority claim for this application (10/002,690) based on provisional application 60/23,336 is denied because the USPTO received 10/002,690 12 months and 7 days after the filing of 60/23,336; making the arrival of 10/002,690 7 days past the due date. However, the mailing of 10/002,690 on or about November 18, 2001, was made at a time when all USPS correspondence to Washington DC based government offices was delayed significantly, because of the post 9/11 anthrax scare. The applicant's recollection is that this delivery took three weeks. The applicant assumed that the USPTO would have given special consideration to correspondence mailed around that time. There was no prior indication to the applicant that there was a problem with this. The first notification that priority was denied, came 2 years and 9 months after the filing of 10/002,690, making rapid action on the part of the applicant in regards to documenting and correcting the problem very difficult. The applicant is requesting that this be re-considered; a letter documenting some of the mail delays is included.

3,4.) The examiner requested the prior art references be placed on the information disclosure form and not in the specification. The information disclosure statement (USTPO PTO/SB/08B or 1449B) has been filled out, and is included with the revised

application. In addition, prior art references have been removed from the specification. Copies of the relevant sections of all prior art are included.

5-7.) The examiner noted a number of problems in the specification:

5.) The examiner noted that certain of the figure sections were not referred to adequately,

6.) a complete minor error check was requested, and

7.) a request to defer from using trademarks was made.

To correct these, the specification has been re-drafted, and substitute drawings submitted, to correct these and a number of problems. The applicant attests that the re-drafted specification contains no new matter. In particular, the previous brief description of the drawings was not truly brief. This section has been changed so that all descriptions of drawings consist of one to two sentences. The previous detailed description of the invention did not refer to the drawings in the extent that it should. To correct this, the previous in depth description of the drawings has been added to the detailed description of the invention. References to the drawings are now made throughout the detailed description of the invention, and fit in with the detailed description to more adequately explain the invention. All parts of all drawings are now referred to. In addition, the examiner noted that in the claims, no correlation step was shown so as to teach the use of the invention to measure ligand binding. The detailed description of the invention with its integrated reference to the figures also has been supplemented with additional statements, to more fully explain how the information from the standards is used to obtain the weight amount of ligand bound in the unknowns. Again here, no new matter

has been added, simply a further explanation of what was previously in the application. The title of the invention has been changed to make it shorter, and to emphasize that the ligand is detected after it is applied to a material (the membrane support) distinct from the binding surface. This is referred to as the secondary immobilization.

Three substitue drawings are submitted: the schematic drawing figures: figures 1, and 3, have been re-done, to better explain the invention and show how the cells/lysates/standards/gels and membrane interact. These are similar to the previous figures 1 and 3, but with additional lanes represented, so that these schematic figures could be matched up with others that show actual scans of films. All of the information in these substitute figures was presented in the original specification so that no new matter is presented. A substitute drawing of figure 2 is also submitted. This displays a more complete analysis of the Tf binding to cells, with all samples displayed: cell bound, competitive bound, unbound, and standards. In this new figure, the reduction of the data to a Scatchard analysis is presented so that the use of the invention for this is visualized. This type of use was discussed in the original specification, and does not represent new matter. The assay for the new figure 2 was performed in September of 2001.

The examiner noted a possible use of the invention in the prior art of Cavanaugh, et al.(1998). The previous embodiment of the invention describing DNA binding particularly matched this prior art. To correct this, the applicant desires that the drawing outlining a schematic of this DNA ligand binding (Figure 5), to be removed from consideration (canceled). In reviewing the specification, the applicant noted that the

procedure of this DNA binding section did not parallel the other methods presented. Therefore, the paragraph entailing measuring a DNA ligand was shortened and changed. Since the applicant had mentioned the use of measuring DNA binding in the original application, it was thought that continued mentioning of DNA binding as an embodiment, in keeping with the theme of the invention, could be allowed. No new matter has been added, simply that the proposed method for measuring DNA ligand binding has been changed to match the method already described for the other five ligands.

An old version of the specification marked as clearly as possible as to indicate deletions and additions is included, as well as a clean version of the new specification. Thus, the substitute specification should adhere to all requirements of MPEP 608.01(q)

Claim Objections:

8 – 27.) The examiner had noted a number of problems with the claims. Since the correction of these would have produced an undesirably marked up presentation, all previous claims have been canceled, and re-stated as new claims. In addition, the re-drafting of the old claims produced dependent claims which were not grouped near their respective independent claims. In order for the claims to be grouped together in a logical manner, a re-stating all claims as new was required. The new claims precisely parallel the old in content.

The punctuation of all claims has been changed so that there is only one capital letter at the beginning, and one period at the end. All typographical errors have been corrected.

All vague references such as "any other", "other form of", "any other suitable", "other based", or "but not limited to", have been eliminated and replaced with normal patent language.

An itemized list of corrections to the stated claim problems follows:

8.) The examiner noted punctuation errors in claims 36-49, and 45-46. These have been corrected in the new claims.

9.) The examiner noted inappropriate definitions in claims 36 and 38. The definition of all items in all claims is now of the "an X comprising of" for the first mention and "said X" or "the X" for subsequent mentions.

10.)"The process as claimed in claim" was noted as inappropriate in claims 37-49. All references to process have been changed to method, with dependent claims using "the method of claim X".

11.) and 12.) The examiner noted typing errors in claims 37, 38, 45, 46, 48, and 49, which have been corrected.

13.) The examiner noted the use of "but is not limited to" was noted as inappropriate. All uses of this phrase have been eliminated.

14.) The examiner noted typing errors in claims 42 and 46, which have been corrected.

15.) The examiner noted that old claims 43 and 44 were essentially duplicates. In the new claims, the method that these old claims refer to have been united into one claim.

16.) The examiner objects to the use of the "any other", "other form of", "any other suitable", and "other based" phrases used in certain of the claims, as the specification does not teach how to recognize or use the items described by these. The examiner notes that the use of these phrases essentially result in the claims claiming more than what the specification describes. To correct this, all claims have been re-stated to use the "comprises", "is comprised of", "includes", and "is selected from a group consisting essentially of" phrases, so that the claims use patent language, and refer only to broad and specific elements that the specification mentions.

17.) The examiner noted that the claims do not logically guide one as to how all the steps teach ligand binding or internalization. To correct this, the use of standards and the signals from standards has been more clearly delineated and stated as the correlative step where one can ascertain the amount of ligand in the unknowns. Also,the major independent method claim now refers to applying the ligand to a known amount of surface. Thus, the method is knit together in a way so as to indicate how to measure amount of ligand bound per unit of surface. Statements added to the new specification to more clearly define the use of standards help to indicate the correlation.

18.) The examiner object to the use of the "but is not limited to" phrase in claims 37-47. To correct this, in the new claims, all uses of this and other indefinite phrases have been eliminated.

19.) The examiner object to the use of the "any other" or "other forms" phrases in claims 38-40, 42, 44, and 46. To correct this, in the new claims, all uses of this and other indefinite phrases have been eliminated.

20.) The examiner objects to the use of non-patent language in mentioning alternate elements in claim 38-41. To correct this, all claims have been re-stated to use the "comprises", "is comprised of", "includes", and "is selected from a group consisting essentially of" phrases, so that the claims use patent language, and refer only to broad and specific elements that the specification mentions.

21.) The examiner objects to the use of the "any other suitable blotting matrix" phrase in claim 42. To correct this, in the new claims, all uses of this and other indefinite phrases have been eliminated.

22.) The examiner objects to the use of the "other based detection" phrase in claims 43 and 44. To correct this, in the new claims, all uses of this and other indefinite phrases have been eliminated.

23.) The examiner objects to the mentioning of avidin and streptavidin in claim 44, as the method outlined in the major independent claim (old claim 36) is not stated in a way as to provide an antecedent for the use of these reagents. These agents were not mentioned in the original specification. To correct this, the new claims refer to such elements in a broad sense as: "an agent which specifically detects the labeled ligand" or "an agent which specifically detects the previous agent", so as to avoid the use of an element not included in the specification.

24.) The examiner objects to the use of the "such as" and "etc." phrases in claim 45. To correct this, in the new claims, all uses of this and other indefinite phrases have been eliminated.

25.) The examiner objects to the mentioning of "the final antibody's" and "avidin" and "streptavidin" in claims 46 and 47, as the method outlined in the major independent claim (old claim 36) is not stated in a way as to provide an antecedent for the use of these reagents. To correct this, the new claims refer to such elements in a broad sense as: "an agent which specifically detects the labeled ligand" or "an agent which specifically detects the previous agent", so as to avoid the use of an element not included in the specification.

26.) The examiner objects to the use of the "varied conditions" phrase in an unspecified claim. To correct this, in the new claims, all uses of this and other indefinite phrases have been eliminated.

27.) The examiner objects to the use of the "the same" phrase in claims 48 and 49; as these are dependent claims, the methods that they refer to need not be mentioned, and referring to these methods as "the same" is indefinite. To correct this, in the new claims, all uses of this phrase has been eliminated.

28.) The examiner rejects all claims as the invention is incomplete due to the existence of a gap between the steps. This gap consists of a lack of a correlative step which recites how to evaluate a biological ligand. To correct this, the use of standards and the signals from standards has been more clearly delineated and stated as the correlative step where one can ascertain the amount of ligand in the unknowns. Also,the major independent method claim and the specification now refer to applying the ligand to a known amount of surface. Thus, the method is knit together in a way so as to indicate how to measure amount of ligand bound per unit of surface (i.e.: per cell or per mg cell lysate protein). Statements added to the new specification to more clearly define the use of standards help to indicate the correlation.

29.) The examiner notes that methods mentioned in the prior art, in an article by Cavanaugh and Nicolson.(1998) teach the methods of the current invention. The applicant draws attention to the fact that the new claims now require the use of standards in the method. The strength of the invention is the ability of users to calculate the weight amount of ligand in unknown sample bands on the blot matrix, by comparison to signals

obtained from known amounts of the ligand (standards) applied to the membrane. Both methods in the prior art example do not and could not be standardized.

The first method cited in Cavanaugh and Nicolson is that of immunofluorescent staining of cell surface transferrin receptor using a phycoerythrin-labeled antibody to the receptor. Here, the anti-transferrin receptor antibody acts a ligand for the transferrin receptor, and the cells act as the binding surface, which contain said receptor.

However, in Cavanaugh and Nicolson the bound phycoerythrin-labeled antibody is detected by flow cytometry. To match the invention, Cavanaugh and Nicolson would have had to solubilize, or make a lysate of the phycoerythrin-labeled anti-transferrin receptor antibody-cell combination, electrophorese the lysate in conjunction with standard quantities of the ligand, blot to a membrane, and detect the antibody using a detectable conjugated anti-phycoerythrin. Then, they could have used the signals from the standard quantities of phycoerythrin-labeled anti-transferrin receptor antibody to construct a standard curve, so as to accurately quantitate the weight amount of antibody originally bound to the cells. As presented, Cavanaugh and Nicolson use flow cytometry and can only display relative differences in ligand binding by the different cells.

The second method cited in the prior art of Cavanaugh and Nicolson which approximates the invention, is that of affinity isolation of biotinylated transferrin receptor. Here, the labeled ligand is not a preparation of a pure labeled ligand, but a complicated mixture of the entire population of all biotinylated cell surface constituents. This is applied to an

immobilized receptor: transferrin-agarose (the surface) which will hopefully recognize only one of the biotinylated entities in the mixture (the transferrin receptor). The ligand mixture is applied to the surface, ligand-surface complex is washed, bound components released(solubilized), separated by electrophoresis, blotted, and detected using a labeled avidin/streptavidin. A major band at the expected size of the preferred ligand was seen and presumed to be that only that transferrin receptor ligand. However, there was no guarantee of that. Other biotinylated cell surface components of similar molecular weight, which bound to the receptor (surface) would also be present in this band. In addition, because the labeled ligand exists in a potpourri of other biotinylated entities, and no pure ligand exists, it is impossible to standardize the system and calculate the amount of putative ligand bound to the receptor. Again as presented, Cavanaugh and Nicolson can only display relative differences in biotinylated transferrin receptor content in the biotinylated cell surface solutions from the different cells. With this assay, the reagents are reversed from the one that the patent describes. Here, the ligand preparation is a mixture of unknown labeled entities, and the insoluble surface a characterized population of single receptors. In the preferred embodiment of this patent application, the ligand exists as one defined pure entity, and the insoluble surface to which it binds is an unknown of multiple receptors, one of which binds the pure ligand.

Other claim remarks:

The applicant has left certain of the claims open, or perhaps slightly broader than what the specification dictates. For secondary immobilization, the specification mentions "membrane support". Since this does not cover use of materials obvious in the prior art,

such as polystyrene plates, claim 52 refers to this as a "support" with the hopes that the coverage in claim 63 could be allowed, where the support includes protein binding materials. Likewise, the membrane detection agents in the specification only refer to antibodies. However, this does not cover recognizing agents used commonly in the prior art, such as avidin/streptavidin. The specification has always mentioned the use of a biotin-DNA ligand, but refers to detecting this with anti-biotin, and does not mention avidin/streptavidin. The use of avidin/streptavidin is common in the prior art, so claim 52 and 67 refer to the ligand recognizing materials as "detection agents", with the idea that use of non-antibody agents could be allowed. Also, alkaline phosphatase is a common enzyme used in detection systems, in the prior art. The specification only mentions horse radish peroxidase for this. So, it was thought that claim 71 could be allowed where the detection agent moiety includes conjugated enzymes. Text to cover these could be added in a future specification amendment, it the examiner allows.

The references and differences with the present invention:

Samuel *et. al.* (1988), discusses the sensitive detection of FITC-labeled protein on Western blots using an anti-FITC antibody, however, this is not directed towards any particular assay.

Haselbeck *et al.*, (1990) discuss the detection of Western blot-localized digoxygenin labeled proteins using anti-digoxygenin, however in this case, the blot itself serves as the binding surface, where a glycoprotein is immobilized to a membrane, then a digoxygenin labeled lectin is used as a ligand to detect particular carbohydrate groups on the

membrane-situated glycoprotein. The lectin is then detected using anti-digoxygenin. The receptor bound ligand is not solubilized and blotted independently, nor is standardization using blotted known amounts of digoxygenin labeled lectin discussed.

In a reference manual, Haselbeck and Hosel (1992) discuss methods similar to the above in using labeled ligands to detect certain types of groups on glycoproteins and proteins immobilized to blots; via the use of anti-label antibodies. However, the receptor bound ligand is not solubilized and blotted independently, nor is standardization using blotted known amounts of labeled ligand discussed.

Cavanaugh, et al. (1999) teach methods similar to Cavanaugh and Nicolson (1988), as discussed above, in the response to examiner point 29. Both of these references are mentioned also as examples of traditional binding studies using radiolabeled ligand.

Gordon (1995) discusses the measurement of the binding of FITC-Concanavalin A and radiolabeled Concanavalin A to cell surfaces. A comparison of the use of both types of label is reported. The reference uses flow cytometry to perform all measurements for the FITC labeled Concanavalin A, and uses a complicated absorbance technique was to calculate FITC- Concanavalin A protein bound to cells, to provide data for a Scatchard analysis

Palupi (2000) discusses the measurement of the binding of FITC-β-lactoglobulin to cell surfaces. The reference uses flow cytometry to perform all measurements for the binding

and uses a complicated fluorescence channel analysis technique was to calculate FITC-β-lactoglobulin protein bound to cells, to provide data for a Scatchard analysis.

Neidergang (2000) discusses the measurement of the binding of FITC-Convulxin to cell surfaces. The reference uses flow cytometry to perform all measurements for the binding and uses a complicated fluorescence FITC-calibration bead technique was to calculate FITC- Convulxin protein bound to cells, to provide data for a Scatchard analysis.

In contrast to the above three, the invention of this application does not use flow cytometry, and standardization is easily achieved by simply applying or transferring known amounts of ligand protein to the detection membrane support.

The Inoue, et al. (1993), ,and LaGrange, et al. (1993) references serve as backgrounds for the analysis of ligand binding using a radiolabeled ligand.

Schaffer(1994) discusses the binding of radiolabeled insulin to the insulin receptor. This is accomplished by the invention of this application, using a non-radiolabeled insulin.

Zhang, et al. (1997) reports on the use of FITC-Annexin V binding to serve as an indicator of apoptosis. All of the analysis was by flow cytometry or immunofluorescence microscopy. LeGall, et al. (2000) report on the shortcomings of cell trypsinization and cell removal in the measurement of apoptosis by the use of Annexin V binding. In contrast to these, the invention of this application does not use flow cytometry,

standardization is easily achieved, and cellular binding of Annexin V does not require removal (trypsinization) from the plates.

US patent 5,962,223 entails the binding of two DNA probes to a target which contains a contiguous sequence recognized by the probes. One of the two probes contains a label. The other has an option of having a hook. After binding to the target, all un-reacted probes are removed. The bound probes are then ligated together into one; this is then released by high temperature treatment, collected, and the label detected. An option is to affinity collect the unified probe on a support which specifically recognizes the hook. Presumably, the support is collected and the label detected. However, the claims don't clearly delineate this. The process differs from the application because it deals strictly with DNA ligands; it requires two ligands to bind to the surface; these two must be covalently linked to each other on the binding surface, and there is no stated method for standardization. In the 5,962,223 embodiment closest to this application, a label and a hook are required on the bound entity whereas in this application, a label suffices.

US patent 6,027,890 and 6,815,212 entail the binding of a first tagged member to a biological sample which contains a second member that binds the first member. Unbound first member is washed from the sample. The tag on the bound first member is then cleaved off, collected, and detected by non-fluorescent spectrometry, or potentiometry. This is different from the application in that the tag is cleaved from the ligand, and the detection method is restricted.

US patent 5,576,436 discusses the formulation of a new class of fluorescent labeled cell binding ligands. These are claimed to be more sensitive than others, so as to permit direct ability to measure amounts of these on the surface to which they bind. Thus, their use as materials which can take the place of radiolabeled ligand is possible. The detection technique of fluorescent spectrophotometry is mentioned. The applicant questions the use of fluorescent spectrophotometry when dealing with cellular or tissue material. These samples can often produce significant interference. With the current invention, interfering material is washed away or separated out. In addition, the current invention does not use fluorescent spectrophotometry.

Request for constructive assisstance

The applicant has amended the specification, drawings, and claims so that they are proper and define a novel non-obvious method. If this application is not believed to be in condition for allowance, the applicant respectfully requests the constructive assistance and suggestions of the examiner pursuant to MPEP 2173.02 and 707.07(j), in order so that the applicant can submit an allowable application as soon aspossible.

Very respectfully,

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In the Drawings:

1.) Drawing number 5 has been canceled.

2.) Substitute drawings for figures 1-3 are enclosed.

3.) The numbering for all other drawings has been altered, to account for the cancellation of figure 5.

4.) References to the drawings in the substitute specification have been amended so that all sections of all drawings are referred to.